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MINISERIES/SPECIAL ARTICLE

The Role of CXC Chemokines in the Regulation of Tumor Angiogenesis

Sergio Dias, Ph.D., Margaret Choy, B.A., and Shahin Rafii, M.D.*

Division of Hematology/Oncology, Weill Medical College of Cornell University, New York, New York

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THE CHEMOKINE FAMILY

Chemokines comprise a small group of secreted, heparin-binding proteins containing four highly conserved cysteine residues. These cytokines have a typical triple-stranded, β-sheet three-dimensional structure (1) and have been classified based on the positions of their N-terminal cysteine residues. The CC chemokines, which represent the majority of chemokines identified to date, are characterized by the presence of two adjacent cysteines at the amino terminal end. On the other hand, CXC chemokines have a single nonconserved amino acid separating the two N-terminal cysteines (see reference 2 for an updated review on the structure of chemokines).

FUNCTIONS OF CHEMOKINES

Chemokines are primarily known for their capacity to induce leukocyte recruitment and activation in processes that require active cell migration, such as inflammatory responses, bacterial or viral infections, and wound healing (3–6). Other functions of chemokines have been described more recently, particularly for the CXC chemokines, and include their ability to regulate angiogenesis directly—through interaction with their receptors on endothelial cells (7)—or indirectly, by attracting inflammatory cells which release angiogenic factors such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) (8,9).

Members of the CXC chemokine subfamily exhibit angiogenic or angiostatic properties, based on the presence or absence of a structural and functional m tif, which led to the creation of a novel chemokine classification. CXC chemokines can be subdivided based on the presence of the ELR (Glu-Leu-Arg) motif, which precedes the first cysteine residue on the N-terminus of these chemokines (10). ELR$^+$ chemokines are generally potent neutrophil chemoattractants with pro-angiogenic proper-

* Corresponding author. Shahin Rafii, M.D., Division of Hematology/Oncology, Room C-606, Weill Medical College of Cornell University, 1300 York Avenue, New York, NY 10021. Fax: (212) 746-8866; E-mail: srafii@mail.med.cornell.edu

Table 1

Examples of CXC Chemokines with Angiogenic (ELR⁺) or Angiostatic (ELR⁻) Properties

ELR⁺ Chemokines	ELR⁻ CXC Chemokines
IL-8	PF-4
ENA-78	IP-10
GRO-α	MIG
GRO-β	SDF-1
GRO-γ	
GCP-2	

IL-8, interleukin 8; ENA-78, epithelial neutrophil activating protein-78; GRO-α, growth related gene-α; GCP-2, granulocyte chemotactic protein 2; PF-4, platelet factor 4; IP-10, interferon inducible protein; MIG, monokine induced by interferon-γ; SDF-1, stromal cell-derived factor-1.

ties, whereas CXC chemokines, which lack the ELR motif (ELR⁻), are generally monocyte and T-cell chemoattractants with potent angiostatic properties (2,10) (Table 1).

CELLULAR PATHWAYS INVOLVED IN TUMOR ANGIOGENESIS

Tumor growth requires sustenance of established endothelium, as well as proliferation of new blood vessels, a process referred to as angiogenesis (11–13). Formation of new blood vessels facilitates rapid and timely delivery of nutrients and oxygen, as well as removal of waste products; these processes, in turn, enhance tumor proliferation and sustenance. By releasing endothelial survival and growth factors, tumor cells promote neo-angiogenesis, which supports their own growth and determines the size of the tumor mass.

Tumor angiogenesis results from the balance between endothelial cell apoptosis and proliferation (14,15). Tumor cells, by elaboration of a wide variety of stimulatory factors including VEGF and FGFs, promote proliferation and suppress apoptosis of endothelial cells. Therefore, inhibition of stimulatory signals and/or induction of apoptotic signals will result in a decrease in endothelial cell mass with a concomitant decrease in tumor cell mass (15).

CXC CHEMOKINES AND PHYSIOLOGICAL/"NORMAL" ANGIOGENESIS

The strongest experimental evidence supporting a role for chemokines in normal angiogenesis comes from wound healing studies. It is well established that angiogenesis is required for efficient scarring and healing f wounds, a process that involves recruitment and activation of inflammatory cells.

Previous studies demonstrated intense chemokine expression in wounds, and showed a correlation with the recruitment of inflammatory cells. For instance, monocyte chemotactic protein-1 (MCP-1) has been implicated in mast cell infiltration (16), whereas macrophage inflammatory protein-1α (MIP-1α) attracts macrophages to the wound healing site (17,18). By attracting proinflammatory cells that can release angiogenic growth factors, chemokines may thus contribute to the neovascularization process required for efficient wound healing.

Elevated chemokine levels have also been detected in processes that require physiological/normal angiogenesis, such as the menstrual cycle. It was shown that levels of MCP-1 are highest premenstrually, and decrease with the increase in estrogen levels as the cycle starts (19). Finally, chemokine levels are elevated and correlate with an abundant leukocyte infiltrate in the mouse uterus during pregnancy (20).

Given their role as potent leukocyte and other inflammatory cell chemoattractants, and the pro-angiogenic properties of these cells, it is not surprising that chemokines may be involved in physiological/normal angiogenesis. However, few studies have tried to address these issues, and further experimental evidence is required to conclude whether chemokines play a crucial role in regulating physiological angiogenesis.

CHEMOKINES AND TUMOR ANGIOGENESIS

In contrast to the lack of experimental data on chemokines and normal angiogenesis, there is ample evidence supporting a role for chemokines in tumor/malignant angiogenesis. Chemokine expression, particularly for the CXC chemokines containing the ELR motif (ELR⁺), is increased in a variety of tumors, and correlates with a more invasive (metastatic) and more vascularized (angiogenic) tumor phenotype (Table 2). A list of cancer types, the most abundant ELR⁺ CXC chemokine detected, and the phenotypic changes in the tumors associated with increased chemokine expression are shown in Table 2. These studies have suggested that the level of expression of ELR⁺ CXC chemokines may regulate diff rent aspects of tumor biology such as tumorigenesis, the onset of me-

Table 2

The Role of ELR⁺ CXC Chemokines in Regulating Different Aspects of Tumor Biology; Shows Different Cancer Types, the Chemokine Detected at Highest Level in the Tumor, and the Change in Tumor Phenotype Associated with Such Expression

Type of Cancer	Chemokine	Phenotype	Reference
Melanoma	IL-8	Angiogenesis, tumor growth, metastasis	21
Gastric carcinoma	IL-8	Angiogenesis, tumorigenesis	22
Pancreatic cancer	IL-8	Tumorgenesis, metastasis	23
Head and Neck	IL-8	Large primary tumor	24
NSCLC	IL-8	Angiogenesis	25
NSCLC	ENA-78	Tumor progression	26
Ovarian carcinoma	IL-8	Tumor progression	27
Prostate	IL-8	Tumor progression	28
Mesothelioma	IL-8	Tumor progression	29
Glioblastoma	IL-8	Tumor progression	30

tastasis, and the acquisition of a more angiogenic phenotype.

As noted in Table 2, most studies identified interleukin-8 (IL-8) as the main pro-angiogenic chemokine expressed in human tumors. Therefore, initial reports on the role of chemokines in tumor angiogenesis focused mostly on IL-8. It was first shown that IL-8 had the capacity to mediate endothelial cell chemotaxis, proliferation, and induce angiogenesis in vitro and in vivo (31,32). Note that these effects were observed in the absence of inflammation, suggesting a direct effect on endothelial cells.

A role for IL-8 in tumor angiogenesis was subsequently investigated in tumor models in vivo. Neutralizing antibodies against IL-8 blocked the growth and formation of metastasis by human prostate and lung tumors in severe combined immunodeficiency syndrome (SCID) mice (33). Other studies have reinforced the importance of IL-8 in regulating tumor growth. For instance, transfection of human gastric carcinoma (34) and melanoma (35) cells with IL-8 increased angiogenesis, tumorigenesis, and formation of metastasis in nude mice. In melanoma cells, increased IL-8 expression was shown to regulate the activity of matrix metalloproteinase-2 (MMP-2), which may explain the increased metastatic behavior of the transfected cells (36). Furthermore, in pancreatic cancer, IL-8 is regulated by acidosis and hypoxia, and an increase in its expression is correlated with a more tumorigenic and metastatic behavior of human pancreatic cancer cells in nude mice (23).

Note that IL-8, as for other CXC chemokines, has been suggested not to act directly on the tumor cells, suggesting that its tumor growth-promoting effects involve an increase in angiogenesis. However, expression of IL-8 receptors has been detected both on tumors as well as endothelial cells in breast cancer biopsies (37), whereas human colonic epithelium also expresses chemokine-related receptor-4 (CXCR4), a receptor for stromal cell-derived factor-1 (SDF-1) (38). Furthermore, despite experimental evidence suggesting that the angiogenic effects of IL-8 are exerted at the endothelial cell level, neither IL-8 binding nor IL-8-induced calcium flux was demonstrated on human umbilical vein or dermal microvascular endothelial cells and, even by reverse transcriptase-polymerase chain reaction (RT-PCR), IL-8 receptors could not be detected on these cells (39).

ELR⁻ CXC CHEMOKINES BLOCK TUMOR ANGIOGENESIS

As mentioned above, ELR⁺ CXC chemokines such as IL-8 stimulate angiogenesis. Indeed, it has been suggested that the presence of this amino acid ELR motif may confer the angiogenic nature of chemokines. This hypothesis received further support from studies which demonstrated that ELR⁻ CXC chemokines such as interferon inducible protein-10 (IP-10), platelet factor-4 (PF-4), and monokine induced by interferon (MIG) have angiostatic properties both in vitro and in vivo (2,32,40,41).

Furthermore, in order to establish whether the presence of the ELR motif was critical for the angiogenic or angiostatic role of CXC chemokines, site-directed mutagenesis was used to insert amino acid residues from IP-10 or PF-4 into the ELR motif of wild-type IL-8 (32). Conversely, using a similar approach, a mutant MIG protein containing the ELR motif immediately adjacent to the first cysteine in its primary structure was created. As predicted, the mutated ELR⁻ IL-8 had angiostatic activity, whereas the mutant MIG produced positive angiogenic responses both in vitro and in viv (32).

These initial studies have also shown that ELR⁻ CXC chemokines block endothelial cell chemotaxis and are ac-

tually capable of blocking the angiogenic effects of IL-8, epithelial neutrophil-activating protein (ENA)-78, and bFGF. The angiostatic action of these chemokines was demonstrated in established in vivo angiogenesis assays such as the corneal micropocket assay (42,43), and growth factor-induced neovascularization of subcutaneously implanted Matrigel plugs in nude mice (44). In the corneal micropocket assay, implantation of pellets containing chemokines such as IL-8, ENA-78, growth-related protein (GRO)-α or granulocyte chemotactic protein (GCP)-2, VEGF, or bFGF produced positive angiogenic responses (42,43). When combined with IP-10, MIG, or SDF-1, the angiogenic properties of ELR$^+$ CXC chemokines, VEGF, or bFGF were markedly abrogated (45). Because there was no evidence of increased inflammation, these results suggested that the angiogenic and/or angiostatic effects of CXC chemokines were produced independent of their role in leukocyte chemoattraction.

Given the powerful in vitro and in vivo angiostatic effects of ELR$^-$ CXC chemokines, namely IP-10 and MIG, there has been considerable interest in exploiting these properties in tumor models as a way to block tumor angiogenesis. Intratumor administration of recombinant IP-10 was shown to decrease tumor growth and angiogenesis in mice bearing aggressive adenocarcinomas (45), and in non-small cell lung cancer-bearing mice, higher tumor and plasma IP-10 levels correlated with a decrease in tumor incidence and delayed tumor progression (46). Also in a model of human non-small cell lung cancer, overexpression of the ELR$^-$ CXC chemokine MIG resulted in decreased angiogenesis and tumor growth (41).

It was suggested from these studies that by modulating the level of angiogenic versus angiostatic chemokines at the tumor site, it might be possible to decrease or block tumor angiogenesis and delay tumor growth. This may be achieved by systemic or local delivery of angiostatic chemokines, or alternatively by administration of agents known to modulate chemokine expression. Subsequently, monitoring the angiogenic versus angiostatic chemokine levels in tumors may give a prediction of the therapeutic outcome.

This approach has been employed in preclinical studies using the antitumor cytokine IL-12. In models of murine breast cancer (47) and human lymphoma (48), the levels of the antiangiogenic chemokines IP-10 and MIG correlated with decreased angiogenesis, delayed tumor growth, and in some cases, cure after IL-12 therapy.

CHEMOKINE RECEPTORS AND ANGIOGENESIS

Although most cell types produce different chemokines, chemokine receptors have considerable ligand specificity profiles. This is particularly striking for the ELR$^+$ CXC angiogenic chemokines (which bind CXCR-2) versus the ELR$^-$ CXC angiostatic chemokines, which bind CXCR-3 (see Fig. 1; reviewed in references 2 and 49). IL-8 does, however, also bind CXCR-1, and SDF-1 binds CXCR-4 (2,50). This apparent receptor specificity in angiogenic versus angiostatic chemokines has received further support from studies on the pathophysiology f Kaposi's sarcoma (KS).

The KS-associated herpesvirus (KSHV) is detected in all KS biopsies, and has been implicated in the path genesis of KS (51). In particular, certain oncogenic proteins encoded by the virus have been suggested to confer the angiogenic phenotype seen in KS lesions. One such protein, encoded by the viral ORF74, is a G-coupled receptor

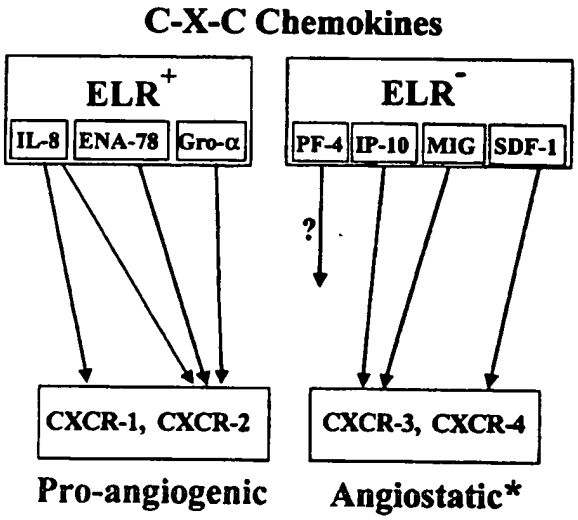

Figure 1. Some of the CXC chemokine family members, divided according to the presence or absence of the ELR motif in their primary structure, their receptor specificity, and pro-angiogenic or angiostatic properties. IL-8, interleukin 8; ENA-78, epithelial neutrophil activating protein-78; GRO-α, growth related gene-α; PF-4, platelet factor 4; IP-10, interferon inducible protein; MIG, monokine induced by interferon-γ; SDF-1, stromal cell-derived factor-1. ?: The receptor for PF-4 has not been identified. *Although SDF-1 is an ELR$^-$ chemokine, there is little evidence supporting an angiostatic versus a pro-angiogenic function.

which stimulates intracellular signaling leading to proliferation, and has been shown to induce an angiogenic phenotype when transfected into normal cells (52). Note that this KSHV-G-protein-coupled receptor has a high degree of homology with CXCR-2 (52).

Given their receptor-binding specificity, the angiostatic chemokines IP-10 (and possibly MIG) have also been suggested to represent unique members of the chemokine family. In addition to CXCR-3, these chemokines bind a specific heparin sulfate proteoglycan-associated receptor on endothelial cells, exerting their angiostatic activity by inducing cell cycle arrest (53).

GENE TARGETING OF CHEMOKINE AND CHEMOKINE-RECEPTOR GENES

Despite significant evidence supporting a role for chemokines in angiogenesis, chemokine as well as chemokine receptor-deficient mice have not been reported to have any major angiogenesis defects. Actually, there is an absence of chemokine knock-out studies documenting its effects on angiogenesis in normal or pathological conditions. The CXCR-4 (receptor for SDF-1) knock-out mice provide the exception, because these mice have hematopoietic deficiencies, cardiac defects, as well as defective vascularization of the brain (54). Finally, IP-10-overexpressing transgenic mice have defective post-wound healing neovascularization (55), highlighting the angiostatic properties of this chemokine described in other models.

CONCLUSIONS

A considerable amount of evidence supports a role for chemokines in tumor angiogenesis, whereas for physiological/normal angiogenesis, further experimental evidence is lacking. In tumors, the expression and activity of ELR^+ CXC chemokines may also regulate tumor progression and the formation of metastasis. These properties of chemokines may provide a basis for antitumor strategies aimed at blocking chemokine production or secretion by tumor as well as accessory cells. A diagram showing some of the CXC chemokines, and the ELR^+ (pro-angiogenic) and ELR^- (angiostatic) members and their receptors can be seen in Figure 1.

Finally, given the apparent specificity of pro-angiogenic versus angiostatic chemokine receptors, it is legitimate to hypothesize that these may also have different downstream signaling pathways. Inhibitors for specific signaling intermediates or strategies to block receptor function may thus have antiangiogenic as well as antitumor effects.

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